

Virulence genes in *Staphylococcus aureus* isolated from cell phone screens of dentistry students in Cuenca-Ecuador

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ABSTRACT. *Staphylococcus aureus* is one of the main pathogens of importance in health care. Distributed worldwide, it has considerable impact on community and nosocomial infections. This bacteria has the *tst* and *lukS-F PV* genes that code for the TSST-1 toxin and leucocidin. These pathogenic microorganisms have the ability to survive for long periods on inert surfaces such as mobile phone screens. Senior dentistry students work in clinics and use their cell phones often, which could potentially spread this pathogen. We looked for the *tst* and *lukS-F PV* genes in *S. aureus* isolated from mobile phone screens of final year dentistry students. This was a descriptive cross-sectional observational study using 92 samples from the mobile phone screens of dental students, among which 16 were positive for *S. aureus*. They were identified by means of culture methods and detection of the *nuc*, *nucA* and *femB* genes. The DNA was extracted using the alkaline lysis method; PCR was used in the molecular identification of the *tst* and *lukS-F PV* genes. Nine of the 16 *S. aureus* isolates had the *tst* gene, and 1/16 the *lukS-F PV* gene. This study shows the high frequency of virulent *S. aureus* on the

mobile phone screens of dental students, which may contribute to the spread of this important pathogen.

Key words: *Staphylococcus aureus*; Virulence factors; Leukocidins; Toxic shock syndrome; Cell phones

INTRODUCTION

Currently, *Staphylococcus aureus* is one of the main pathogens of importance in health care. Distributed worldwide, the impact of community and nosocomial infections is significant. It can induce a wide range of infections, adapt, and proliferate in various environmental conditions, it is the human pathogen with the highest capacity for resistance to antibiotics. It is important to understand its molecular microbiology, this can help to manage the clinical epidemiology of your infections, the proper diagnosis, and the correct prevention for their control. (Gonzalez and Perez, 2017). Bacteria such as *S. aureus* has been shown to survive seven weeks to seven months on inert surfaces. Factors that contribute to the survival of this bacterium have been evidenced, such as temperature and high humidity. (Hernandez et al., 2017). Devices with screens like mobile phones have non-biological surfaces that act as inanimate vectors so that certain pathogenic bacteria found in hospital areas are transmitted and at the same time contaminate health personnel; since by not being aware of this reality, they transmit -and at the same time- self-contaminate on contact with the skin because they do not always wash their hands with the proper frequency or with the appropriate disinfectants (Hernandez et al., 2017).

Healthcare workers use mobile phones to share X-rays, lab reports, and EKGs. This improves the quality of care, especially during emergencies. Although the use of mobile phones in hospitals has many benefits, it is still a major source of contamination. This is because, when in use, mobile phones generate heat, which provides the right conditions for the replication of the bacteria present in them. (Shiluli et al., 2021)

Staphylococcus aureus is the leading cause of septicemia, osteomyelitis, endocarditis, toxic shock syndrome, pneumonia, food poisoning, and furuncle. These problems are more common in patients with a weakened immune system. It is found in the front of the nose in children and adults; in a proportion of 20 to 40% in healthy individuals. The phone is contaminated through hands, saliva, runny nose, etc. of a person who is a carrier of *S. aureus*, the number of bacteria on the hands is higher in people who work in hospital sectors. (Safdari et al., 2020)

It is well documented that *S. aureus* strains produce a variety of protein toxins, including Panton-Valentine leukocidin (PVL), Exfoliative toxin (ET), enterotoxins, Toxic Shock Syndrome Toxin 1 (TSST-1), hemolysins, coagulase, etc. (Mehrotra and Wang, 2000) (Shrestha, 2013). The *tst* gene codes for Toxic Shock Syndrome 1 (TSST-1), which constitutes a sequence of extracellular proteins involved with toxic shock syndrome due to multisystemic diseases. (Corredor and Santacruz, 2011). This toxin blocks the chemotaxis of the white blood cells, blocks the reticuloendothelial system, and incites the inhibition of T lymphocytes (González et al., 2018).

Toxic shock syndrome has clinical evidence, such as skin rash, scaly feet and hands, muscle failure, throat congestion, gastrointestinal symptoms, and rapidly progressing acute kidney failure. The aforementioned clinical symptoms are due to the presence of exotoxins

of bacterial origin that act as superantigens in the human body, emphasizing TSST-1. (Bertelloni et al., 2015). *S. aureus* encoding this toxin is commonly found in many sites including surgical wounds (surgical toxic shock), tampons (menstrual toxic shock), lungs (flu-related toxic shock), peritoneal dialysis catheters, epidermis, as well as mucous membranes (recalcitrant desquamative syndrome) (Bertelloni et al., 2015).

The *lukS-F PV* genes encode for a Pantone-Valentine leukocidin toxin (PVL) that causes alteration of cell permeability, leukocyte destruction (Waleed, 2019). PVL is a specific exotoxin of *S. aureus* that exhibits leukocyte toxicity in neutrophils and macrophages, causes diseases ranging from simple skin and soft tissue infections to invasive diseases such as pneumonia, severe sepsis, and necrotizing fasciitis. (Romero et al., 2016).

PVL was recently associated with community-based methicillin-resistant *S. aureus* (c-MRSA). (Romero et al., 2016). C MRSA positive for Pantone Valentin has a high probability of affecting health due to high bacterial resistance to common antibiotics. (Prevost et al., 1995). The strains that synthesize the aforementioned toxins are more virulent and therefore more dangerous and can put human health at risk. (Corredor and Santacruz, 2011)

The purpose of this research was to look for the *tst* and *lukS-F PV* genes in *S. aureus* strains isolated from mobile phone screens in last-year dental students from 2020-2021.

MATERIAL AND METHODS

Research type and design:

In this research, the non-experimental descriptive method was utilized. The design in this study is observational and cross-sectional. The variables are qualitative: the level of study of the students and the genes that code for TSST-1 and PVL components S and F in *Staphylococcus aureus* strains. During the sampling, the participants were not allowed to clean the screen of their mobile phone with substances that could alter the actual results of the present analysis.

Sample

In this research, samples were taken from the screens of mobile phones that belonged to undergraduate students of the Faculty of Dentistry (they attend the dental clinic) of the University of Cuenca, Ecuador. 92 cell phone screen swab samples were collected between September-December 2020.

For the inclusion of this study, the mobile phones had to belong to undergraduate students of the Faculty of Dentistry and sign the informed consent. Those students who do not agree to participate in the research and do not sign the informed consent are excluded from this study. It is necessary to mention that since a cell phone is an inanimate object, it was not necessary the approval of an institutional human research ethics committee to take samplings from the participants' phones.

Samples were collected using swabs (sterile) with trypticase soy broth (CST), which was rubbed on the cell phone screen (off) by placing it in a test tube containing CST. This

research was carried out in the Genetics and Molecular Biology laboratory of the Center for Research, Innovation and Technology Transfer (CIITT) of the Catholic University in Cuenca.

Methodology

The samples were incubated for 24 hours at 35-37 C in aerobiosis before bacterial analysis. It was inoculated on salty mannitol agar and incubated aerobically for 24-48 hours, at a temperature between 35-37°C. After incubation, mannitol fermenting colonies were selected; subsequently, with these colonies, a colored smear was conducted using the Gram's method. If positive Gram cocci were observed in clusters, the definitive identification was made through genotypic and phenotypic tests. Genotypic identification was performed utilizing the genes *nucA*, *femB* (Hamdan et al., 2015), and *nuc* (Depardieu et al., 2004), specific genes of *S. aureus*. The phenotypic analysis of *S. aureus* was carried out by fermentation in mannitol agar, as well as positive reactions to the tests of coagulase, catalase, and Deoxyribonuclease (DNAse) (Andrade and Orellana, 2019).

Extraction of DNA

The alkaline lysis method was employed to extract DNA from *Staphylococcus aureus* strains, which was performed as follows: *S. aureus* colonies were suspended in 1 mL of distilled water, then it was centrifuged at 3,000 rpm for 10 minutes, it was discarded to the supernatant, 50 µL of lysis solution formed by sodium dodecyl sulfate (1% SDS) in 0.25N sodium hydroxide was added, after mixing it, it was kept boiling for 15 minutes, later 450 µL of free water was added nucleases and centrifuged for 20 seconds. Finally, the DNA obtained was stored at a freezing temperature.

Genotypic detection of Toxic Shock Syndrome toxin (TSST-1) and Panton-Valentine (PVL components S and F)

For the molecular detection of the *tst* and *lukS-F PV* genes, which code for TSST-1 and PVL components S and F, the PCR technique was used; the primers (primers), size of the amplicon, ATCC® strains, and the PCR conditions are detailed in Table 1. The PCR reactions for the two aforementioned genes were performed with 10 µl of Promega GoTaq Green 2X Mastermix, 1.5 µL of each primer, 5 µL of ultrapure water, and 2 µL of the extracted DNA, giving a final volume of 20 µL.

These amplification reactions were conducted in an Agilent Sure Cyclor 8800 thermal cyclor. The PCR products were separated by electrophoresis on 2% w / v agarose gels (50ml of gel with 2ul of SYBR Safe DNA Gel Stain 10000x from Invitrogen) on a horizontal chamber immersed in TAE 1X buffer with a protocol of 70V, 70A, and 50W for 60 minutes. The size of the amplicons was calculated according to the migrations in the agarose gels, comparing it with the migration of the standard DNA bands of the molecular weight marker (100 bp Plus DNA ladder) Trackit from Invitrogen and they were photographed on a transilluminator with a digital camera.

Table 1. Primers, amplicon size, ATCC strains and PCR conditions for the identification of the *tst* and *lukS-F PV* genes. (Jarraud, Mougel, Thioulouse, et al., 2002) (Lina, Piemont, Godail-Gamot, Bes, et al., 1999).

Toxins	Genes	Primers (5'-3')	Amplicon size	ATCC Strains	PCR condition
TSST-1	<i>tst</i>	F: TTCACTATTTGTAAGAGTGCAGACCCACT R: TACTAATGAATTTTTTATCGTAAGCCCTT	180 pb	43300	5 minutes at 94°C 30 cycles: 30 seconds at 94°C 1 minute at 55°C 1 minute at 72°C 10 minutes at 72°C
PVL components S and F	<i>lukS-F PV</i>	F: ATCATTAGGTAATGTATGGACATGATCCA R: GCATCAASTGTATTGGATAGCAAAGC	433 pb	25923	

Statistical analysis

Both this study and its results were conducted using absolute and relative frequencies. To this end, no subgroups of the samples were created since the aim was to determine the overall presence of *S. aureus* in the cellphone screens of dental school students. The absolute risk with a 95% confidence interval was calculated using Excel software.

RESULTS

In this research, the *tst* and *lukS-F PV* genes coding for TSST-1 and PVL toxins S and F components were identified from 16 *S. aureus* samples isolated from the cell phone screens of senior students at the University of Cuenca. 9/16 samples of *S. aureus* had the *tst* gene, 1/16 had *lukS-F PV* gene.

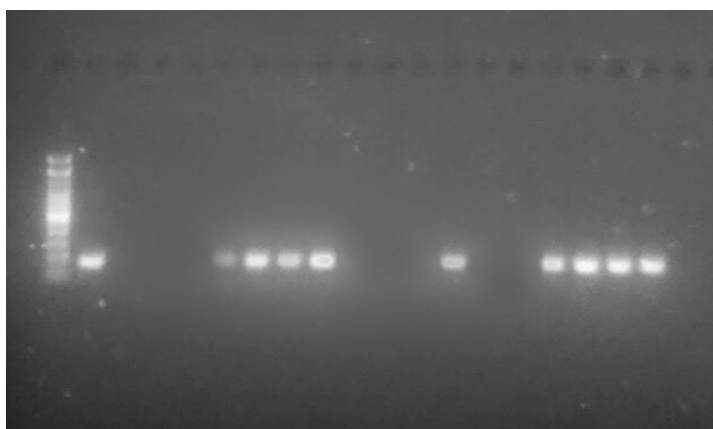


Figure 1: Result of electrophoresis of amplicons for the *tst* gene (180 bp), in *Staphylococcus aureus* taken from cell phone screens, first lane Ladder, second lane positive control *Staphylococcus aureus* strain ATCC 43300, Lane 3 negative control *Streptococcus pyogenes* strain ATCC 12344, Positive samples 4, 13, 32, 38, 48, 58, 64, 65, 68. Negative samples 2, 3, 39, 40, 42, 53, 54 for *tst* gene.

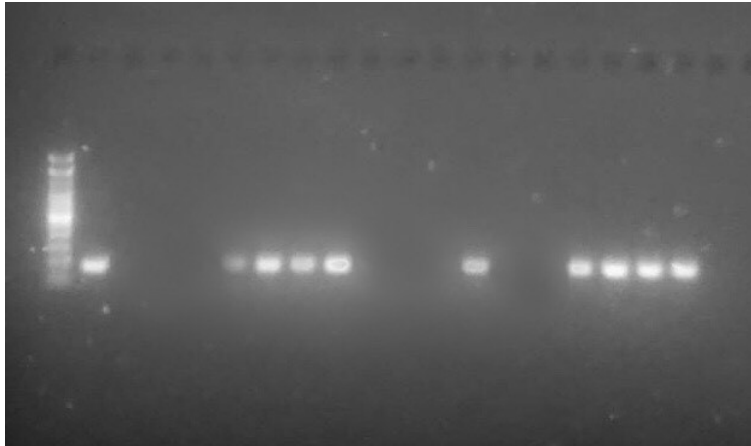


Figure 2: Result of electrophoresis of amplicons for the *lukS-F PV* gene (433 bp) in *S. aureus* isolated in cellphone screens, first lane Ladder, second lane positive control *Staphylococcus aureus* strain ATCC 25923, third lane negative control *Streptococcus pyogenes* strains ATCC 12344, Lane 4 sample # 2 positive, Lane 5 to 19 negative samples for the *lukS-F PV* gene.

DISCUSSION

In this research, the *tst* genes were detected in 9/16 and *lukS-F PV* in one of 16 strains of *S. aureus* isolated from mobile phones of last year dentistry students from the University of Cuenca-Ecuador, which shows that these devices are a source of contamination, a fact that is supported by Katsuse et al (2017) and by Singh et al. (2010); it shows that mobile phones serve as reservoirs of infection in the medical and dental care environment respectively.

The *S. aureus* contamination rate in cell phones in our study was 16/92 (17%), a percentage lower than reported by Muñoz et al (2012) in Mexico (38.7%) and Jansen et al (2019) in Brazil (32%). In a study made by Ulger et al, 39 articles published to review different studies on the relationship between mobile phones and bacterial cross-contamination between 2005 and 2013 were analyzed. Of these, 26 identified *S. aureus*, which validates the importance of the study of these bacteria in these devices. Haun et al (2016) conducted a study in which the contamination of these devices by *S. aureus* was found.

There is very little research performed for the detection of *tst* and *lukS-F PV* in mobile phones, so we will compare with strains of *S. aureus* isolated from other sources. In their study, Cataldo et al (2014) in Paraguay analyzed 112 swab samples from the oral cavity of children, of which 33% were positive for *S. aureus*; none were positive for *lukS-F PV*.

González et al (2018) analyzed in Colombia, 95 samples positive for *S. aureus* isolated from the nostrils and hands in their study, of which 23% gave a positive result for the *tst* gene; while we found 9/16 to have this gene.

Corredor and Santacruz (2011), conducted an investigation in Colombia in which 100 strains isolated from clinical samples of *S. aureus* were evaluated 7% of the samples were positive for the *tst* gene.

Hait et al (2014) analyzed 85 samples positive for *S. aureus* isolated from environmental samples and raw ingredients in the United States, of which 55% were positive for the *lukS-PV* gene. Cobos et al. (2010) conducted a study in which 329 positive strains for methicillin-resistant *S. aureus* isolated from clinical samples were analyzed, in Spain, of which 11% were positive for *lukS-PV*.

Sila J. et al. (2009) in the Czech Republic analyzed 200 samples of *S. aureus* isolated from blood and nasal samples; the *tst* gene was present in 18% of blood samples and 22 of nasal samples.

Tst, the gene for toxic shock syndrome toxin-1 (TSST-1), is a potent superantigen and is the most common cause of toxic shock syndrome. This toxin is produced exclusively by *S. aureus*, and approximately 20% of natural isolates are producers. (Lindsay et al., 1998; Deurenberg et al., 2005).

On account of the results obtained in this research, the constant monitoring of this pathogen in these devices and the determination of virulence genes for infection control is of great importance. (Romero et al., 2016). Given the small sample size, it is not possible to generalize the current results to the entire population, but it is possible to obtain an incidence of the *tst* and *lukS-F PV* genes in *S. aureus* strains taken from samples of cell phone screens of students from last year of dentistry.

CONCLUSIONS

We found a high percentage of virulent strains of *S. aureus* (carriers of the *tst* and *lukS-F PV* gene) on the mobile phone screens of dental students, which may contribute to the dissemination of this important pathogen. Thus, it is essential to implement prevention measures such as the disinfection of mobile phones to avoid the spread of virulent strains of *S. aureus*.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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